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CONTRIBUTIONS FROM THE DEPARTMENT OF BOTANY
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SEXUAL FUSIONS AND SPORE DEVELOPMENT OF THE FLAX RUST

By FRED D. FROMME

NEW YORK
1912

Reprinted, without change of paging, from Bulletin of the Torrey Botanical Club 39:
113-131. *pl. 8, 9.* 13 Ap 1912

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[From the Bulletin of the Torrey Botanical Club, 39: 113-131, *pls.* 8, 9. 17 Ap 1912.]

Sexual fusions and spore development of the flax rust

Fred D. Fromme

(with plates 8 and 9)

The variations found in different species of rusts so far investigated as to their sexuality, have made evident the desirability of further investigating a large number of forms, if we are to arrive at a definite understanding of the sexual processes in the Uredineae in general. From this standpoint I have taken up the study of the flax rust, *Melampsora Lini* (Pers.) Desm. The caeoma type of aecidium has in all cases proved favorable for the study of sexual fusions. The flax rust is no exception in this respect, and the abundance of the fusion stages in my material leaves little to be desired. Dr. E. W. Olive suggested the problem to me, and the results here recorded were largely worked out under his direction at Brookings, S. Dak.

A summary of the literature prior to 1908 has been given by Olive ('08) and still more recently by Maire ('11), so that the later papers only will be discussed here. While the sexual nature of the fusions in the rusts may be regarded as definitely established, the morphological character of the two cells involved and the phylogenetic significance of the process are still points of contention. Two more or less distinct types of fusion have been recognized. (1) A "partial cell fusion" where fertilization is effected by the migration of the nucleus of a vegetative cell into a special "fertile cell," as found by Blackman in *Phragmidium violaceum*. (2) A complete cell fusion between equal gametes, as figured by Christman in *Phragmidium speciosum* and suggested for *Uromyces Caladii* and *Caeoma nitens*. This type of fusion was later substantiated by Blackman in *Melampsora Rostrupi*, and by Olive in *Triphragmium Ulmariae* and *Gymnoconia interstitialis* (*Caeoma nitens*). Both of these processes Blackman considers as reduced types of fertilization that have supplanted a true fertilization, in which the spermatia functioned as male cells and

the "sterile cells" above the "fertile" or female cells as trichogynes. On this view the Uredineae are derived from the red algae. Christman considers the "fusion of equal gametes" as a true fertilization in which a "non-resting zygospore" is produced. He suggests that the nuclear migrations of Blackman may be pathological in nature like similar migrations observed by him in the teleutosorus of *Puccinia Podophylli*. The sterile cells are merely "buffers" and the spermatia may be degenerate gametophytic conidia. Christman has also found a fusion of equal cells in the formation of the primary uredospores in *Phragmidium Potentillae-canadensis*, thus showing the morphological equivalence between the primary uredospores and aecidiospores.

Olive, in his 1908 paper, described a further series of forms: *Triphragmium Ulmariae*, *Gymnoconia interstitialis* (*Caeoma nitens*), *Phragmidium Potentillae-canadensis* and the microform *Puccinia transformans*. He also studied nuclear divisions in three other species: *Uromyces Scirpi*, *Uromyces Lilii*, and *Puccinia Cirsii-lanceolati*. *Triphragmium Ulmariae* has a crustlike primary uredosorus very similar to that of *Phragmidium Potentillae-canadensis*, studied by Christman. In this form Olive finds numerous cases of wide open cell fusions of the type described by Christman. The fusion pores, however, may be of varying diameters. In most cases they are broad, the intervening cell walls being entirely absorbed, but occasionally the pore is narrow so that the nucleus is constricted somewhat in passing. He found no cases, however, where the nucleus is drawn out in a fine thread in passing through an imperceptible pore as figured by Blackman. He further observed, that although the fusing cells may be placed side by side in the same plane, as Christman found them, one of the gametes is perhaps more often found to lie somewhat below the other. In such instances only the upper of the fusing cells appears to have cut off a sterile cell. When the two gametes are not in direct contact a very short conjugation tube may apparently be formed (*fig. 26*). In all cases figured in *Triphragmium*, only one of the fusing cells enlarges and becomes the basal cell. This method of forming the basal cell is quite different from that described by Christman for *Phragmidium Potentillae-canadensis*, where the fusing cells combine to form the basal cell.

The subsequent budding off of the primary uredospores in a fashion similar to that described by Christman confirms his evidence that the primary uredospores and aecidiospores are morphologically equivalent. Although Christman did not find the fusion stages in *Caeoma nitens*, his material being rather old, Olive figures two cases (*fig. 35* and *36*) for this form, where the fusion occurs between cells placed adjacent and parallel, similar to Christman's figures of *Phragmidium speciosum* but without showing sterile cells. He also figures a third case where the nucleus of one cell is passing into a cell placed immediately above it but belonging to a distinct hypha. This as he points out is very similar in appearance to Blackman's ('04) *fig. 66* and *68*. *Fig. 34* and *36* were drawn from the same section and were but a short distance apart. This instance, in conjunction with his observations on *Triphragmium*, convince him that Blackman's "nuclear migrations" and Christman's "fusion of equal gametes" may occur in the same rust and in the same pustule. Sexual fusions may begin through a pore which is narrow at first so that the nucleus is constricted in passing. Later this pore may broaden and the entire contents of the two cells fuse. He would distinguish between such cases and cases of undoubted pathological migrations such as he finds between the multinucleated cells of the aecidium of *Puccinia Cirsii-lanceolati*. In the typical microform, *Puccinia transformans* on *Tecoma stans*, fusion is shown in the teleutosorus between the end cells of two hyphae in one instance, while in another the end cell of one is fusing with the penultimate cell of the other. The fusions here are immediately followed by growth which results in teleutospores borne on several binucleated cells. The sporophyte generation in this case consists, then, of only a few, three or four, cells. In *Uromyces Scirpi* and *Puccinia Cirsii-lanceolati*, as well as in some eight other species, he found one or more large multinucleated cells at the base of the young aecidium cups. He is inclined to interpret these as sporophytic cells in which nuclear division has proceeded faster than cell division, due possibly to the stimulus to growth derived from the sexual act. He suggests the possibility that the solution of the problem as to the development of the aecidium cup with its peridium and apparent centralized structure may be found in these multinucleated cells. Es-

pecially striking were the cases found in *Puccinia Cirsii-lanceolati*. Fifteen nuclei in a single cell were figured in one instance. From his study of *Triphragmium* and other forms, Olive concludes that only one of the gametes ordinarily bears a sterile cell and that one gamete generally lies somewhat below the other. This leads him to believe that the two gametes differ somewhat in the time of their development. The first hyphae to form the upright layer under the epidermis do not fuse among themselves but cut off sterile cells and are fertilized by the tip cells of hyphae that push up later from below. Olive does not differentiate these cells as "fertile and vegetative," as Blackman has done, but believes they are entirely equal in size and contents and differ only in time of development. He agrees with Christman that the sterile cells are merely "buffers" and cannot be considered phylogenetically as trichogynes.

In two shorter articles, published earlier in the same year ('08), Olive calls attention to the similarity between the multinucleated cells, which he found at the base of a number of young aecidium cups, and the archicarps of De Bary, Massee, and Richards. He thinks it quite probable that the basal cells of the aecidium are the ultimate branches of these multinucleated cells. The cup type of aecidium was probably derived from the more simple caeoma type. He has found as many as six nuclei in a fusion cell of the caeoma type, due probably to nuclear division proceeding faster than cell division, and points out that a still further development of such a cell coupled with partial suppression of other neighboring cell-fusions, especially in a deep lying caeoma, might give rise to the cupshaped type of aecidium.

Kurssanow ('10) reinvestigated *Puccinia Peckiana* (*Caeoma nitens* of Christman and *Gymnoconia interstitialis* of Olive). He agrees with Christman in all essentials. The conjugation is between entirely similar gametes. Sterile cells are normally formed from both conjugate cells, but these have degenerated or have been lifted off by the rupture of the epidermis before the time of fusion. These are mere "buffer" cells and cannot be interpreted as trichogynes. Kurssanow does not accept Olive's attempt to harmonize the observations of Blackman and Christman. Either the two methods of conjugation are limited to the

different forms studied, or Blackman's "partial cell fusions" must be regarded as pathological in nature. Kurssanow has also observed undoubted pathological migrations in his material aside from the normal conjugations.

Maire ('11) has reviewed the numerous problems in the study of the Uredineae and given a résumé of our present knowledge of the cytology of the group. He regards the sexual fusions as well established for forms possessing an aecidium. In reexamining his preparations of *Puccinia Bunii*, an opsis form, he has been able to find the isogamous fusion of two cells to bring about the formation of the primary "synkaryocyte" but is not able to see the stages clearly. He does not regard the question of the isogamous or heterogamous nature of the fusion as of great importance and thinks cases may be found of the union of sister cells or even confirmation of the method of forming a binucleated cell originally described by Sappin-Trouffy and later by Maire, in the microform, *Puccinia Liliacearum*. Maire holds that the present sexual fusions have replaced a primitive sexuality analogous to that in the red algae, in which the spermatia functioned as male cells. The interpretation of the sterile cell as a trichogyne he considers as extremely hypothetical. He holds that the Uredineae and the Basidiomycetes have a common origin, but the latter have perhaps lost all trace of sexuality. He favors the view that the primitive rust forms had spermatia and teleutospores only, and that the aecidiospores and uredospores have been intercalated in the life history to provide for rapid distribution.

Olive, in a recent paper ('11), has also discussed the character of the primitive rusts and the origin of heteroecism. He also holds to the theory that the more complex types were derived from the simpler lepto- and microforms by a progressive development of the sporophyte. This is substantiated by analogy with the higher plants. The primitive forms were probably autoecious. The alternation of hosts was made possible by the production of aecidiospores which are invigorated by the stimulus derived from the sexual act and therefore better able to make the transition to a new host than the uninucleated sporidia. This would mean that the present hosts of the gametophytic stage of the rusts (aecidium), of the heteroecious forms, were the hosts of the original

autoecious ancestors. The prevalence of heteroecious forms with pleophagous sporophytes and the fewness and evident close relationship of the hosts of the aecidial stages in these cases, as well as the multiplicity and remote relationship of the hosts of the sporophyte, support this conclusion.

Melampsora Lini is quite abundant on cultivated flax, *Linum usitatissimum*, in the vicinity of Brookings, S. Dak. A considerable quantity of rusted flax straw was collected in the fall of 1910 and was exposed during the winter in a cloth bag suspended from a window. In the spring a small plot was sown to flax and the rusted straw scattered over the plot. This was watered frequently and numerous infections were obtained, the sori appearing when the young plants were about five inches high. These sori are quite small and it is difficult to distinguish spermogonia from aecidia. Small portions of the leaves and stems were fixed in Flemming's medium fixing solution, imbedded, sectioned, and stained with Flemming's triple stain and Bendas' iron haematoxylin. Sections of the uredo- and teleutosori were kindly furnished me by Dr. Olive from material previously prepared by him.

Melampsora Lini is an autoecious eu-form, i. e., the complete series of spore forms, spermatia, aecidia, uredo, and teleuto, are all borne upon the common host, flax. The aecidium is of the caeoma type, which has been found most favorable for the study of cell fusions, and the teleutospores show the nuclear fusions very clearly.

SPERMOGONIA

These are rather inconspicuous in color and occur on both sides of the leaves and occasionally on the stems. They are produced only by infections with sporidia. They are typically flaskshaped, without ostiolar filaments and are placed subepidermally. Sometimes the spermogonium is merely a diffuse layer of spermatiophores without a definite flasklike structure. Several of these spermogonia may occur in the same localized area but they are seldom confluent. Their frequent close association with the aecidia will be discussed in connection with the development of the aecidium. The spermatiophores from which the spermatia are abstricted arise from large rectangular cells which are arranged in a regular series at the base of the spermogonium. The sper-

matiophores differ from those described by Blackman in that they are divided into a number of uninucleated cells, usually four, each of which puts out a fingerlike process from its upper end on the tip of which a single spermatium is produced. See FIG. 1 and 2. The spermatiophores of *Gymnosporangium clavariaeforme* and *Phragmidium violaceum*, as described by Blackman, are single elongated, uninucleated cells. The single nucleus divides successively to form the nuclei for a number of spermatia which are abstricted from the elongated fingerlike tip of the spermatiophore. FIG. 4 shows two spermatia fully formed while a nucleus still remains in the base of each cell. This seems to indicate that two or more generations of sporidia are produced from the same cell of the spermatiophore.

AECIDIUM

The aecidium arises from a uninucleated mycelium, which in the vegetative condition cannot be distinguished in any way from that which produces spermogonia. It is confined chiefly to the intercellular spaces of the host. The nuclei at this stage are rather small but exhibit a clearly defined chromatin network and a definite nucleole. After a period of vegetative development the filaments grow up between the cells of the mesophyll and reach the epidermis. Here they branch laterally to form a weft of hyphae. The branches next push up vertically and form a sort of palisade of large cells which contain very large nuclei and more compact cytoplasm than the ordinary vegetative cells. See FIG. 5. Each cell now divides somewhat unequally, producing a smaller cell above, which again divides so that two smaller cells are formed above a single larger cell. See FIG. 5. The larger cells are the future gametes and at this stage form a rather even dense layer below the two sterile cells. The shape of the sterile cells is determined by the pressure of the epidermis and that of the surrounding cells. When they press directly against the epidermal cells they are flattened laterally and the upper cell conforms to the outline of the overlying epidermal cell. At other points where the pressure is not so direct they may be somewhat elongated. In either case they soon become vacuolate and disappear usually by the time of sexual fusions. These are the "buffer" cells of Christman, and we see that they are here in two layers instead of a

single layer as he found in *Phragmidium speciosum*. The entire sorus at this time is a group of vertically placed hyphal branches without pseudoperidium or paraphyses. It cannot be considered as a unit in any proper sense but rather as a collection of reproductive units. The development of the sorus proceeds in a more or less centrifugal fashion, the older branches being found at the center while the younger are at the outer borders of the group. The various stages in the degeneration of the sterile cells are well seen in passing from the outer borders toward the center. Christman's interpretation of these sterile cells as "buffers" seems entirely adequate, as their function is evidently a protective one. They relieve the pressure of the epidermis on the underlying reproductive cells and then degenerate to provide room for the subsequent development of the latter. As noted, in all the forms so far investigated only one layer of sterile cells has been observed, while in *Melampsora Lini* two layers of sterile cells are normally produced. In his investigation of *Melampsora Rostrupi*, Blackman occasionally found a double layer of short crushed cells lying above the fused cells, but owing to the age of his material he did not note the method of their formation. In view of the facts above described it seems quite reasonable to assume that Blackman's observation shows that a similar condition is found in *M. Rostrupi*. It is possible that all the Melampsoras develop their gametes at a deeper point in the sorus than the more superficial caeomas of the *Phragmidium* type.

The frequent intimate association between the spermogonia and aecidia, referred to above, is an interesting feature of the flax rust. In rusts bearing both spermogonia and aecidia the former usually precedes the latter in time of development by a period of several days or weeks. In this form, however, a marked difference in time of development does not exist. In general the spermogonia appear somewhat earlier than the aecidia, but they are often found developing simultaneously and intimately associated. Spermogonia may bound the aecidium on either side or may even be included in it. It is very difficult to trace the origin of the mycelium from which the two sori arise on account of the interweaving of the hyphae, but there is no sharp boundary between the two and they cannot be distinguished in the vege-

tative condition. It seems quite probable that they arise from common points and that branches from the same mycelium may form both kinds of sori. The aecidium and spermogonium are frequently separated only by the outer sterile layers of the spermogonium. Their development proceeds simultaneously, spermatia and aecidiospores being formed at the same time and often found lying in the same cavity under the epidermis. Sometimes the spermatia are already present when the aecidium has just reached the stage of sexual fusions. One of the difficulties in assuming that the spermatia are male cells has been the inaccessibility of the cells of the aecidium to fertilization by them and the production of the spermatia prior to the development of the aecidium. Here we have a case, however, where the two develop at the same time, and so near together that fertilization might easily be accomplished.

The large vertical cells of the aecidium now begin to conjugate in pairs. This conjugation is brought about by an absorption of the intervening cell walls at the area of contact. The upper portions of the cells involved are usually in contact so that the absorption takes place in this region. See FIG. 7. The lower portions of the cell walls are usually not absorbed and the fusion cells formed remain with a conspicuous two-legged base. At the time of fusion the cytoplasm of the gametes is quite dense and stains readily, consequently they stand out quite sharply differentiated from the vegetative cells below, which have lost most of their contents. They can readily be distinguished under the low powers of the microscope without the aid of the oil immersion. Sometimes the tips of the gametes converge and the point of contact and absorption is at the center, as in FIG. 8. The area absorbed may be of varying diameters, as Olive holds, but the passage of a nucleus through an imperceptible pore has never been observed in the case of the true fertilizations. Certain cases of nuclear migrations which are apparently of an abnormal nature, will be referred to later. The fusing gametes do not always lie side by side at the same level in the sorus. They may meet at various angles, and frequently one of them lies somewhat below the other. See FIG. 9. I do not believe, however, that in *Melampsora Lini* this indicates a difference in the time of develop-

ment of the gametes, as Olive finds for *Triphragmium Ulmariae*. In the early stages of the sorus the gametes are not always placed in an even parallel layer but may vary in elevation. The irregularities due to the loose indefinite nature of the cæoma quite fully account for the unequal position of the gametes. The presence or absence of sterile cells at the time of fusion, again does not seem a good criterion for determining the relative age of the two gametes. I have observed several cases where both gametes at the time of fusion still have two sterile cells intact. See FIG. 10. As I have pointed out, however, these sterile cells have usually disappeared at the time of fusion.

The abundance of sexual fusions in *Melampsora Lini* is most striking. The sexual fusions figured by earlier students have been scattered and disconnected and apparently only occasionally found. In my material I have sections showing practically every pair of gametes in the sorus in some stage of fusion. These stages are so abundant that there can remain no doubt whatever that the binucleated condition in this form is always instituted by means of a cell fusion between entirely equivalent gametes. FIG. 11 shows a collection of "fusion cells" that were especially well situated for drawing. A triple cell fusion is shown in the center of the group with three ordinary fusions on either side. The "fusion cells" are rather old here, one or more aecidiospores having been produced in most cases, but the double "basal cells" still show the original fusions distinctly. FIG. 12 shows another series of cell fusions. The five pairs of gametes in this instance are of about the same age as those shown in FIG. 11. Younger pairs are shown in FIG. 13. The gametes on the left have just completed the fusion while those on the right have cut off the first aecidiospore mother cell. FIG. 14 shows a mature, binucleated aecidiospore with a chain of intercalary cells and young aecidiospores ending in a double cell fusion below. The process by which the chain of spores and intercalary cells are formed has been fully described and need not be taken up here.

As previously mentioned, Olive has figured a somewhat different method of forming the "basal cell," in *Triphragmium Ulmariae*, from that shown by Christman in *Phragmidium Potentillae-canadensis* and *P. speciosum*. As found by Christman, the

"basal cell" is formed by the equal enlargement of the two fused cells, and the aecidiospore mother cell lies directly over the fused cells. The cases figured by Olive, on the other hand, show that the nucleus of one gamete passes into the other, and the gamete which is now binucleated elongates and functions directly as the "basal cell." Cases of this nature also occur in *Melampsora Lini*. It will be seen in the fusion shown in FIG. 15 that the gamete on the right has elongated and become the "basal cell" and has cut off the first aecidiospore mother cell. The nucleus of the gamete on the left lies in the opening between the two fused cells. A comparison of this figure with FIG. 13 and 14, which are cases of equal contribution to the "basal cell," will illustrate the difference between these two methods. It is interesting to find both of these methods occurring here in the same rust.

Reference has already been made to a triple cell fusion, shown in FIG. 11, which lies in the center of a group of ordinary cell fusions. There can be no question that the condition there shown has been brought about by the fusion of three gametes instead of two. FIG. 16 shows an early stage of a triple cell fusion. The intervening cell walls are absorbed just as in the case of a double cell fusion, and a "basal cell" is produced which contains the nuclei and cytoplasm of three gametes. FIG. 17 is a case of a triple cell fusion where the three-legged base is especially distinct. These triple fusions are of comparatively frequent occurrence in my material. FIG. 18 (PLATE 9) shows a triple fusion which presents a still further complexity. It will be seen from the figure that two adjoining cells in the same filament on the right have fused with a single cell in another filament on the left. This has also resulted in a trinucleated "basal cell" but differs from the usual triple fusions in that two of the gametes come from the same filament. I have observed several other cases of this nature. It seems, then, that not only a single layer of gametes are produced, in this form, but occasionally another cell placed below the ordinary functional cells also has the capacity for effecting fertilization. The three nuclei thus associated in the same "basal cell" probably divide "conjugately" to produce trinucleated aecidiospores. FIG. 19 shows a trinucleated aecidiospore mother cell which has just been formed by the division of the trinucleated "basal cell" on which

it is borne. A later stage is shown in FIG. 20. The trinucleated spore at the top of the chain has cut off a small intercalary cell while the spore mother cell just below is as yet undivided. FIG. 21 shows a mature accidiospore which contains three nuclei. Such spores are frequently found lying free in the cavity under the epidermis among the ordinary binucleated type. A mature accidiospore containing six nuclei was also found. See FIG. 22. This condition might have been brought about by the division of three original nuclei without the formation of the intercalary cell.

Cell fusions between four gametes are sometimes found along with the two- and three-celled types but are relatively infrequent. I have observed only three well-defined cases of this nature. Two of these cases are shown in FIG. 23 and 24. The four cells that have participated in the fusion can be seen distinctly, but the cell contents were stained so strongly that the nuclei could not be distinguished.

Still more striking are the large multinucleated spore mother cells which are occasionally found in the aecidium. One of these, FIG. 25, contains eleven nuclei. The cell is being divided by an ingrowing cell wall near the center. The uneven number of nuclei is probably due to the sectioning, but it is impossible to locate it with certainty on the next section. Another of these large structures is shown in FIG. 26. The evident pairing of the eight chromatin masses indicates that a division of four nuclei is just being completed. The base of the cell is indistinct and I am not able to determine its origin. FIG. 27 shows a case of a completed division of a four-nucleated cell forming a distinctly four-nucleated accidiospore and an intercalary cell in which the nuclei are less distinct but evidently four in number. It seems quite probable that the conditions shown in FIG. 26 and 27 have resulted from an original four-cell fusion. Attention has been called to Olive's observation of large multinucleated cells at the base of certain young aecidium cups especially in *Puccinia Cirsii-lanceolati*. He was inclined to interpret these as sporophytic structures resulting from the greatly stimulated growth following sexual fusions. Whether these are really central organs from which the aecidium develops, has not been established. There is

perhaps some similarity between them and the multinucleated cells that I have found.

I have examined my material carefully to determine whether the binucleated condition is ever brought about by the migration of the nucleus of one cell into an adjoining cell, as described by Blackman, and have found no such cases in the fertile layer of the aecidium, or such as could be considered true fertilizations. Occasionally, however, in the vegetative mycelium below the fertile layer of the aecidium, I have found cases that are very similar in appearance to some of Blackman's figures. One of these is illustrated in FIG. 28. The nucleus here is evidently passing through a very small pore in the walls between two adjoining cells and is drawn out in a very fine thread in the passage. The two cells are small, contain but very little cytoplasm, and lie some distance below the surface of an old sorus which has already produced aecidiospores in the regular manner. Another case of migrations of nuclei is shown in FIG. 29. Two nuclei from neighboring vegetative cells are migrating into the same vegetative cell. It does not seem possible to connect any of these cases with the normal process of fertilization in any way, and the interpretation of Christman, Olive, and Kurssanow of similar phenomena as "pathological" seems most natural.

UREDO

The uredosori arise from a binucleated mycelium which results only from infections with aecidiospores. They are found on both sides of the leaves and on the stems. The sori are indefinite in extent, without pseudoperidium or paraphyses except for a few sterile, capitate filaments, which sometimes occur at the outer borders of the sorus and less frequently are interspersed among the spores within the sorus. The uredospores are borne upon long stalks which are made up of two or three elongated cells. See FIG. 30. They are large, ovate to elliptical in form, and contain two nuclei, as do all of the stalk cells upon which they are borne. The walls are thin and finely and evenly verrucose with low papillae and equatorial, rather indistinct germ pores. The sterile filaments, commonly called paraphyses, which are found among the spores, are long and extremely slender and terminate

in somewhat irregular knoblike cells, which are usually somewhat larger that the uredospores. The walls are somewhat thicker than those of the uredospores and perhaps a little smoother, although the older ones are roughened like the uredospores. Young paraphyses were found to contain two nuclei at the base of the apical knob. See FIG. 30. The stalks were undivided and very slender and contained but very little cytoplasm. The nuclei evidently degenerate very rapidly, as in the older filaments they have disappeared entirely. Of the cytoplasmic contents only a few shriveled threads remain. The nature of these sterile filaments is perhaps not entirely clear, but the observation that they are binucleated when young suggests that they have a common origin with the functional uredospores.

TELEUTO

The teleutospores appear later in the season than the uredospores and are associated with the ripening of the flax. The sori are found on both sides of the leaves and more frequently on the stems. They are sometimes round and isolated but are more often confluent in long areas, which are reddish brown when young and become quite black when old. The spores are of the elongated one-celled type of the Melampsoras and are cemented together above in a firm, waxy layer. See FIG. 31. They are sessile on the short cubical cells at the base of the sorus, which lose their contents with the development of the teleutospores. The young teleutospores are binucleated but soon become uninucleated through a fusion of the two nuclei into one. Occasionally a secondary layer of teleutospores forms an overgrowth above the first. This is apparently brought about by the growing up of the mycelium around the borders of the primary layer of teleutospores to form a secondary layer of shorter teleutospores above the first.

DISCUSSION

The existence of sexuality in the rusts may be considered as well established for those forms that have the caeoma type of accidium. The cup type of accidium, however, needs much further study, though Christman's figure for *Uromyces Caladii* shows that the fusions there are probably essentially similar to

those in the caeoma type, and Olive's multinucleated cells may be correlated with the more definite form and structure of the cup. The double layer of sterile cells in *Melampsora Lini* may possibly be comparable to the pseudoparenchyma of an aecidium cup and indicate a transition between the more superficial caeoma and the deeper cup type of aecidium. The evidence seems to point to the conclusion that the short-cycled micro- and leptoforms are the more primitive and that such a form as the flax rust with its five spore forms is more highly specialized. It is interesting to note that the less differentiated caeoma type of aecidium can persist in these higher types of rusts.

As to the relationships of the Uredineae with other fungi or algae, the preponderance of evidence at present seems to favor the view that the present sexual fusions are a substitute for a more primitive type in which the spermatia functioned as male cells. The existing sexual processes are certainly of a zygosporic character. The conjugation is betwen two entirely similar cells, which participate equally in the formation of a double cell from which a series of spores are produced. This is of course no proof that the primitive form of sexuality was zygosporic. Blackman, as previously stated, would derive the Uredineae from the red algae. While this connection is as yet doubtful, it is perhaps the most plausible view of the origin of the group.

Although Blackman's observation of a "partial cell fusion" by means of "nuclear migrations" in *Phragmidium violaceum* has never been directly disproved, the indirect evidence shown in the equal cell fusions that have been found in all other aecidia of the caeoma type, so far investigated, leads to the conclusion that this form is somewhat aberrant or that the true fertilization has not been observed. The further observations of "pathological" migrations by other investigators and the existence of very similar migrations between vegetative cells of *Melampsora Lini*, which cannot be regarded as true fertilizations, are striking facts. While the area of absorption between two gametes may be small at first and later broaden, as Olive has shown, there still remains a considerable difference between the passage of a nucleus as a fine thread through an extremely small pore, which cannot be seen before or after the passage, and the passage of a nucleus through

a rather small pore which later broadens to permit the entire contents of the two cells to fuse.

The fusions of three and four cells, which are occasionally found in *Melampsora Lini*, are perhaps to be compared with cases of di- or polyspermy in animals. The first beginnings of triple cell fusions are of rather common occurrence in the group of the Conjugatae, but the complete fertile product of such a fusion is at least a rare occurrence. De Bary figures one case in *Zygnema pectinatum* of a completed conjugation between three cells, but the resulting zygospore has not rounded up, as do the zygospores formed by the fusion of two cells, and remains partially distributed in a horseshoe form between the three fused cells. As I have shown, the triple fusions in the flax rust are functional in the production of trinucleated aecidiospores. It would be very interesting to determine whether these spores in turn produce a mycelium with trinucleated cells. Blackman has also observed trinucleated "basal cells," aecidiospore mother cells, and aecidiospores in *Phragmidium violaceum* but did not determine the manner in which this condition is brought about. He observed that the size of these nuclei is usually somewhat less than that of the normal paired nuclei, and suggests that one of the nuclei of a binucleated cell may have divided while the other remained in the resting condition. He also suggests that a migration of two nuclei into one cell would bring about a similar result. In the short-cycled form *Puccinia Malvacearum*, abnormal vegetative cells and teleutospores containing three nuclei were found by Blackman. Triple and even quadruple fusions are certainly to be reckoned with as widespread and fairly common phenomena in the sexual reproduction of the rusts. They show a certain elasticity in the relations of the gametes, which may be further evidence that the sexual process, as at present found, is secondary and highly modified from the primitive condition in the group.

SUMMARY

The spermatia of the flax rust are produced on septate branching spermatiophores, which differ in this respect from the unbranched non-septate spermatiophores described by Blackman for *Phragmidium violaceum* and *Gymnosporangium clavariaeforme*.

This feature may furnish a further basis for use in the classification of the Uredineae.

The fusing cells in the aecidium are entirely similar and form a fairly even series at the base of the sorus. Two short sterile cells are normally formed above each gamete. Their function is evidently protective and they may correspond to the pseudoparenchyma of young aecidium cups. Sexual fusions are very abundant in this form, and many pairs of gametes in various stages of union can be found side by side in the same preparation.

Fusions of three and four cells have also been found in addition to the normal two-cell fusions and are perhaps to be regarded as further evidence that the sexual processes as found in the rusts are of a secondary character. Large multinucleated cells are also present in the same sorus with two-, three-, and four-cell fusions.

COLUMBIA UNIVERSITY.

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Explanation of plates 8 and 9

PLATE 8

FIG. 1. A branching, septate spermatiophore bearing spermatia on the finger-like elongations of the cells. × 1,500.

FIG. 2. Another branched spermatiophore. The two upper cells have not as yet produced spermatia. The penultimate cell shows the beginning of the fingerlike outgrowth. × 1,500.

FIG. 3. A septate, unbranched spermatiophore. × 1,500.

FIG. 3a. A spermatium. × 2,250.

FIG. 4. A two-celled spermatiophore bearing spermatia. × 1,500.

FIG. 5. A portion of a young aecidium showing a single layer of larger cells, the gametes, with two layers of short sterile cells above and vegetative mycelium below. The upper sterile cells show conformity to the outline of the overlying epidermis. An early stage of fusion is shown between the second pair of gametes from the right. × 1,000.

FIG. 6. A young pair of gametes more highly magnified. Two short, crushed, sterile cells are present above each gamete. × 1,500.

FIG. 7. Cell fusion between two equal and parallel gametes. The upper portions of the intervening cell walls have been absorbed. × 1,500.

FIG. 8. An early stage in the fusion of two gametes. The absorption is at the center of the two cells, as the tips are not in contact. × 1,000.

FIG. 9. One of the gametes lies somewhat below the other. × 1,000.

FIG. 10. Fusion between two gametes, both of which still have two sterile cells intact. × 1,000.

FIG. 11. A group of seven "fusion cells." A triple cell fusion lies in the center of the group with three double cell fusions on either side. One or more aecidiospores have been produced from most of the "fusion cells." × 750.

FIG. 12. A group of five "fusion cells." × 750.

FIG. 13. A pair of younger "fusion cells." The gametes on the left have just completed the fusion while those on the right have cut off the primary aecidiospore mother cell. × 1,000.

FIG. 14. A mature aecidiospore and a chain of intercalary and spore mother cells which have been cut off from the "basal cell" below. × 750.

FIG. 15. Only one of the gametes functions as the "basal cell." The cell on the right has elongated after the fusion and has cut off the primary aecidiospore mother cell. × 1,000.

FIG. 16. An early stage of a triple cell fusion. × 1,000.

FIG. 17. A completed fusion between the tips of three gametes. × 1,000.

PLATE 9

FIG. 18. Another type of triple cell fusion. Two adjoining cells in the same hypha have fused with a single cell on the left. × 1,000.

FIG. 19. A trinucleated spore mother cell borne on a trinucleated "basal cell," which has been formed by a triple cell fusion. × 1,000.

FIG. 20. A trinucleated "basal cell" which has produced the second aecidiospore mother cell. The primary aecidiospore mother cell has divided to form an aecidiospore and intercalary cell. × 1,000.

FIG. 21. A mature trinucleated aecidiospore. × 1,000.

FIG. 22. A mature aecidiospore containing six nuclei. × 1,500.

FIG. 23. A quadruple cell fusion. The "fusion cell" is stained so heavily that the nuclei are not distinguishable. × 1,000.

FIG. 24. Another quadruple cell fusion. × 1,000.

FIG. 25. A large spore mother cell, of the aecidium, containing eleven nuclei. A cell wall is being formed near the center of the cell. × 1,000.

FIG. 26. A cell containing eight paired chromatin masses indicating that a simultaneous division of four nuclei is just being completed. × 1,500.

FIG. 27. A four-nucleated aecidiospore and intercalary cell. × 1,500.

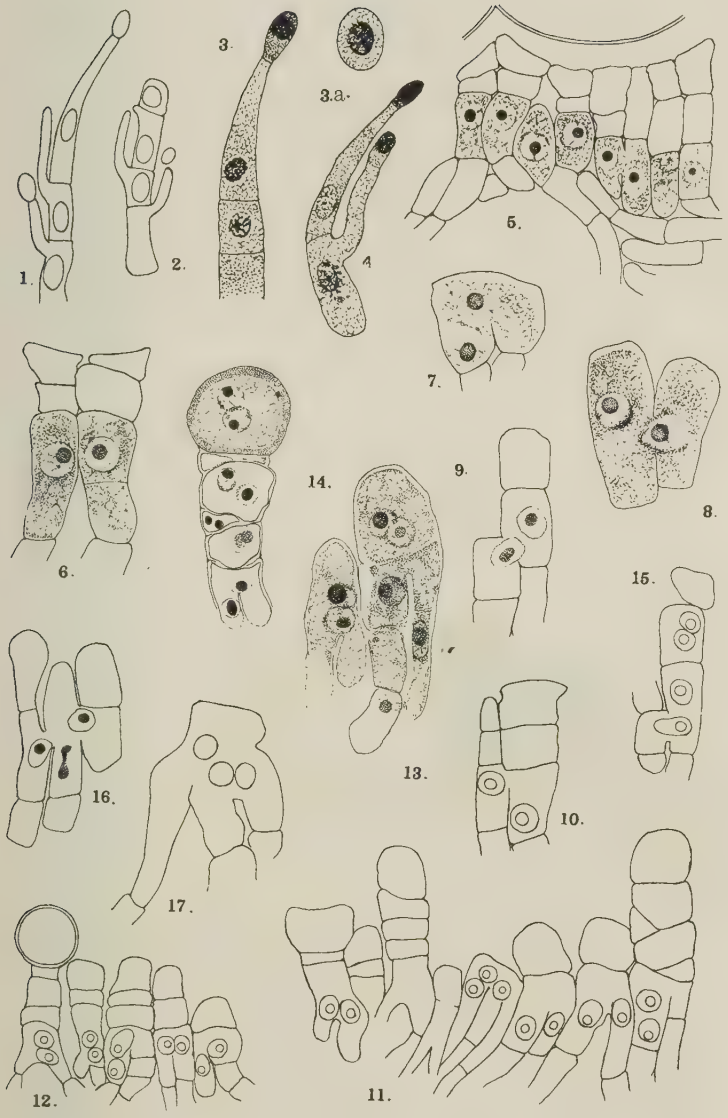
FIG. 28. Migration of a nucleus through the walls of two adjoining vegetative cells which lie below the fertile layer of an old aecidium. × 1,500.

[The nucleus is not as distinct in the plate as in the original drawing, and the disposition of the cytoplasm has been somewhat changed in the reproduction of the figure on the plate.]

FIG. 29. Migration of two nuclei from two vegetative cells into a third vegetative cell. × 1,500.

FIG. 30. A portion of an uredosorus. The binucleated uredospores are borne on long stalks made up of two or three binucleated cells which arise from binucleated mycelium. Sterile, capitate paraphyses are shown at the outer borders of the group. The knoblike apical cell is somewhat irregular in outline and the stalk is non-septate and very slender. The young paraphyse on the right is binucleated and contains a small amount of cytoplasm while the older one on the left has lost its contents. (Slightly diagrammatic.) × 750.

FIG. 31. A portion of a teleutosorus showing the appressed layer of one-celled teleutospores, the tips of which are imbedded in a waxy substance. The spore on the right is binucleated while the others have become uninucleated through a fusion of the primary nuclei. × 750.



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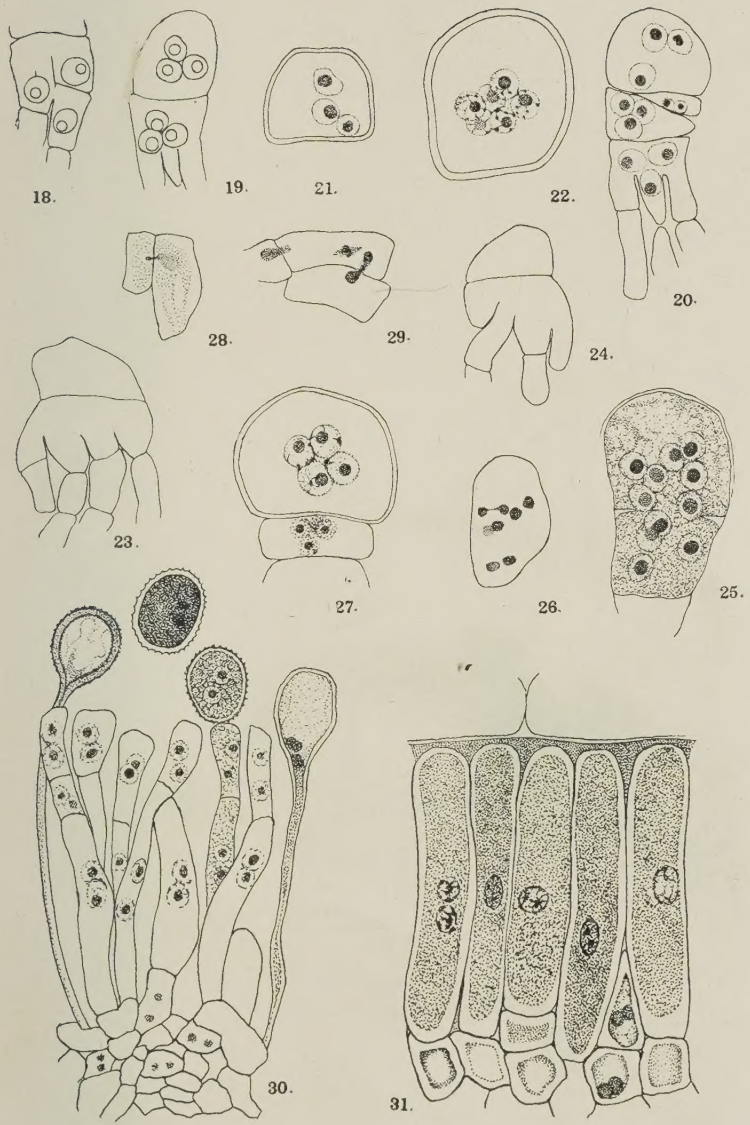

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Professor of Botany.

COLUMBIA UNIVERSITY,
NEW YORK CITY.